The Characterisation of Dynamin-Like Proteins in the Gastric Pathogen *Helicobacter pylori*

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Emma Mary Dawson, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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Signature of candidate:

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Emma Dawson, January 2018

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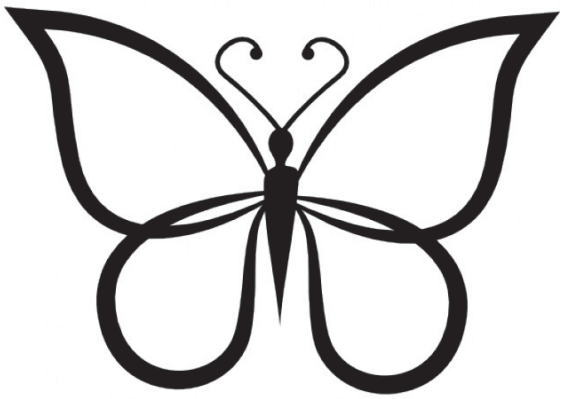

PUBLICATIONS

1. Duggin, I. G., Aylett, C. H., Walsh, J. C., Michie, K. A., Wang, Q., Turnbull, L., **Dawson, E. M.,** Harry, E. J., Whitchurch, C. B., Amos, L. A. and Lowe, J. (2015). "CetZ tubulin-like proteins control archaeal cell shape." Nature, 519 (7543): 362-365.

2. **Dawson, E.M.** (2016) "Dynamin-Like Proteins contribute to membrane integrity and acid tolerance in *Helicobacter pylori".* ASM Syntrophy, 17(7): 7.

CONFERENCE PROCEEDINGS

1. **2012 Oral -** 29th RNSH/UTS/USYD Scientific Research Meeting, Sydney

2. **2013 Oral -** 13th East Coast Bacillus, Sydney

3. **2013 Oral -** 30th RNSH/UTS/USYD Scientific Research Meeting, Sydney

4. **2015 Poster -** 32th RNSH/UTS/USYD Scientific Research Meeting, Sydney

5. **2015 Poster -** BacPath 13, Melbourne

6. **2015 Poster -** 40th Lorne Protein Structure and Function, Victoria

7. **2016 Oral -** Gordon Research Seminar, Vermont, USA

8. **2016 Poster** - Gordon Research Conference, Vermont, USA

9. **2016 Oral** - Becton Dickinson Award
    a. ***Finalist***

10. **2016 Oral** – Sydney Protein Group Thompson Prize, Sydney
    a. ***Awarded the Thompson Prize***

11. **2017 Oral** – BacPath 14, Adelaide

ABBREVIATIONS

Ab (α-)	Antibody
AMP	Ampicillin
BDLP1	Bacterial Dynamin Like Protein1
BLAST	Basic local alignment search tool
BSA	Bovine Serum Albumin
BSE	Basal Signalling Element
bp	Base Pair
DDA	Data dependent Acquisition
Δ	Deleted
Dlp1a, 1b, 1, 2	Dynamin-Like Protein 1a, 1b, 1, 2
DIA	Data independent Acquisition
dlp	Dynamin like protein
DNA	Deoxyribonucleic acid
DynA	Dynamin A
ETEC	Enterotoxigenic *Escherichia coli*
FITC	Fluorescein isothiocyanate
x *g*	Centrifugal force (unit x gravitational field)
g	Gram
GED	GTPase effector domain
GFP	Green fluorescent protein
GTP	Guanosine triphosphate
HP	*Helicobacter pylori*
h	Hour (s)
IFM	Immunofluorescence Microscopy
IgG	Immunoglobulin G
IniA	Isoniazid-induced gene A
Kan	Kanamycin
Kb	Kilo basepair (s)
kDa	Kilodalton (s)
L	Litre (s)
LB	Loading Buffer
LeoCBA	Labile enterotoxin output A, B, C
LT	Heat-labile Enterotoxin
M	Molarity
mA	Milliamp (s)
min	Minute (s)
mL	Millilitre (s)

ms	Milliseconds
MQW	Milli-Q water
MS	Mass Spectrometry
Mut	Mutant
MW	Molecular Weight
n	Nano (10^{-9})
OD	Optical Density
OMP	Outer Membrane Protein (s)
OMV	Outer membrane Vesicle (s)
ORF	Opening reading frame
p	Pico (10^{-12})
PAI	Pathogenicity Island
%	Percent
PBS	Phosphate Buffered Saline
PH	Pleckstrin Homology Domain
PCR	Polymerase Chain Reaction
PRD	Proline-Rich Domain
(m)RNA	(messenger) Ribonucleic acid
RT	Room Temperature
rpm	Revolutions per minute
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	Seconds
SWATH-MS	Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra Mass Spectrometry
TBS	Tris Buffered Saline
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	Tris(hydroxymethyl)methylamine
w	Weight
WCL	Whole Cell Lysate
WT	Wild Type
v	Volume
µ-	Micro (10^{-6})

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ABSTRACT

In eukaryotic cells, it has been well established that large GTPases of the dynamin superfamily are important drivers of membrane curvature and constriction during membrane fusion and fission processes such as clathrin-coated endocytosis (1, 2). More recently, dynamins have been identified in a number of bacteria including *Bacillus subtilis, Nostoc punctiforme* and *Escherichia coli* (3-5). However, despite their putative identification in over 1500 bacterial species to date, minimal understanding of their biological function has been established in bacteria (6). One such bacterial dynamin, a previously characterised enterotoxigenic *E. coli* virulence factor, LeoA has been shown to be involved in outer membrane vesicle formation for the release of heat-lable LT toxin (5, 7). Upstream of LeoA there were two other dynamins identified, LeoC and LeoB, encoding a full-length dynamin only when expressed together. This is a seemingly split ORF, a first for dynamin (5). The closest homologs to these genes are found in the gastric carcinogenic pathogen *Helicobacter pylori* and we have termed them *dlp1a*, *dlp1b* and *dlp2* respective to their LeoCBA homologs. Therefore, this thesis endeavoured to investigate for the first time the presence of dynamin-like proteins (DLPs) in *H. pylori*, an important and surprisingly understudied human pathogen that is known to be dependent on membrane dynamics for virulence (8-10). It is also a pathogen that survives and thrives in one of the harshest environments, the acidic environment of the human stomach, where membrane integrity is essential to its protection.

The investigation into the presence of DLPs in *H. pylori* undertaken in this thesis was achieved through a diverse series of aims. Firstly, a bioinformatic survey undertaken to determine the conservation of the DLP genes in the *H. pylori* genome highlighted conservation greater than 90% in *H. pylori*, unprecedented for any known bacterial dynamins. For the first time, *in vivo* expression of *dlps* was shown in *H. pylori* using detection of the mRNA transcripts as well as the protein products via Western blotting. Interestingly, a single-nucleotide indel was identified in a region of overlap between *dlp1a* and *dlp1b* that differs between sub-isolates of *H. pylori*, leading to either the split or the joined forms of *dlp1* (the full-length gene). Furthermore, the analyses of *dlp1* and *dlp2* mRNA and their protein

products were consistent with this genotypic variation and might represent a novel regulation mechanism.

A preliminary investigation into the biochemical features of the DLPs such as localisation, self-interaction and lipid binding capabilities was commenced. In strains containing the fused *dlp1*, Western blotting and immuno-fluorescence microscopy suggested that Dlp1 is membrane-associated, whereas Dlp2 is approximately equally distributed between the cytosol and membrane. Also, initial studies suggested that Dlp2 may dimerise, interact with liposomes, as well as showing potential for interaction with its operon partner Dlp1a; all fundamental dynamin features. The gene arrangement and presence of a predicted transmembrane helix in Dlp1, similar to the membrane-binding domain of eukaryotic dynamin, suggested that these proteins might act as a hetero-complex involved in bacterial membrane dynamics.

Finally, the previously uncharted function of DLPs in *H. pylori* was explored using both direct and global approaches. Knockout of the *dlp* operon in *H. pylori* caused a high proportion of cells to develop a compromised cell membrane in dye-penetration assays, and minimal capacity to overcome acidic exposure as well as minimal growth in acidic conditions. Utilising a global proteome approach, SWATH-MS, one of the most comprehensive quantitative proteomics study to date of the acid response in wild-type and knock-out strains was achieved. This analysis highlighted the potential role that dynamins may have in acid adaptation, with proteins involved in processes such as motility and cytoplasmic neutralisation detected in higher abundance in the absence of dynamin. Taken together, this suggests that *H. pylori,* with a compromised membrane in the absence of dynamin, is attempting to overcome the higher influx of acid into the cell by "escaping" to conditions that are more neutral.

Overall, this thesis provides the first evidence that this important human pathogen, *H. pylori* utilises membrane remodelling events in some capacity driven, by dynamins, to maintain membrane integrity. A feature that is necessary to ensure continual pathogenesis in its niche environment the stomach of over half the world's population.